Study Title

Approximate Lethal Dose (ALD) by
Skin Absorption of H-21216 in Rabbits

Laboratory Project ID

Haskell Laboratory Report No. 839-95

Author

Tracy A. Filliben

Study Completed On

April 1, 1996

Performing Laboratory

E. I. du Pont de Nemours and Company
Haskell Laboratory for Toxicology and Industrial Medicine
P. O. Box 50, Elkton Road
Newark, Delaware 19714

Medical Research Project No. 10074-001

GENERAL INFORMATION

Substance Tested:	Propanoic acid, 2,3,3,3-tetrafluoro-2-(heptafluoropropoxy)-, ammonium salt
Synonyms/Codes:	• H-21216 • HFPO Dimer • Ammonium perfluoro-2-methyl-3-oxahexanoate • 2,3,3,3-Tetrafluoro-2-(heptafluoropropoxy)propanoic acid ammonium salt • Ammonium Salt • Hazard Code UN2928 • Cost Center ID Code E83640-19
Physical Form:	White solid
Purity:	Greater than 99%
Composition:	Not provided by sponsor
Contaminants:	Not provided by sponsor
Submitter's Notebook No.:	E83640-19
CAS Registry No.:	62037-80-3
Sponsor:	DuPont Fluoroproducts E. I. du Pont de Nemours and Company Wilmington, Delaware
Study Initiated-Completed:	10/30/95 - 4/1/96
In-Life Phase Initiated - Completed:	10/31/95 - 11/14/95

Approximate Lethal Dose (ALD) by Skin Absorption of H-21216 in Rabbits

SUMMARY

H-21216 was applied to the shaved, intact skin of 2 male New Zealand White rabbits at a dosage of 5000 mg/kg. The application site was occluded for approximately 24 hours after which the test substance was removed. The rabbits were observed for clinical signs of toxicity and dermal effects for 14 days following removal of the test substance (weekends excluded).

No deaths occurred, and no clinical signs of toxicity were observed in either rabbit. H-21216 produced moderate erythema in both rabbits by 1 day after application and was still observed 2 and 3 days after application. By 6 days, mild or moderate erythema was observed. Mild erythema persisted from 7 to 10 days. By 13 days after application, the rabbits exhibited slight erythema; all erythema was clear by study completion. No edema was observed during this study. Epidermal scaling and sloughing were observed in both rabbits from 6 to 13 days after application. In addition, one rabbit exhibited a small area of necrosis from 2 to 6 days after application. By 7 days, the area of necrosis had sloughed and an area of alopecia was then observed until study completion.

Under the conditions of this test, the ALD was greater than 5000 mg/kg of body weight. This substance is considered to be, at worst, slightly toxic (ALD between 5000 and 10,000 mg/kg) when applied to the shaved, intact skin of male rabbits.

SIGNATURE PAGE

Report by: Amy L. Williamson
Amy L. Williamson
Secretary

Work by: M. Scott Karr
M. Scott Karr
Toxicology Technician

Reviewed and
Approved for Issue: Tracy A. Filliben 4/1/96
Tracy A. Filliben
Study Director

TF/alw

INTRODUCTION

The purpose of this study was to determine an approximate lethal dose (ALD) by skin absorption of H-21216. The ALD was defined as the lowest dose of the test substance administered that caused the death of a test animal within 14 days after exposure.

MATERIALS AND METHODS

A. Animal Husbandry

Young adult male HM:(NZW)fBR New Zealand White rabbits were received from Hare Marland, Hewitt, New Jersey. The rabbits were housed singly in suspended, stainless steel, wire-mesh cages. Each rabbit was assigned a unique identification number which was recorded on a card affixed to the cage. The last 3 digits of the identification number were written on the inside of each rabbit's ear with a water insoluble marker. The rabbits were offered approximately 125 grams of Purina Certified High Fiber Rabbit Chow® #5325 daily during the study. Water was available ad libitum.

Haskell Laboratory has an animal quality monitoring program. This program is monitored and administered by the Laboratory Veterinarian. Water samples are periodically analyzed for total bacterial counts and for the presence of coliforms, lead, and other contaminants. Additionally, samples from freshly washed cages and cage racks are periodically analyzed to assure adequate sanitation by the cagewashers. Data from this program are maintained separately from study records. Animal feed is certified by the manufacturer to meet specified nutritional requirements and to be free of a list of specified contaminants. On the basis of these analyses, there is no evidence suggesting that contaminants were present in the feed or water in amounts which may have interfered with the results of this study.

Rabbits were quarantined, weighed, and observed for general health for approximately 2 weeks. Animal rooms were maintained on a timer-controlled, 12-hour light/12-hour dark cycle. Environmental conditions of the rooms were targeted for a temperature of 20°C ± 1°C and relative humidity of 50% ± 10%. Excursions outside these ranges were judged to have been of insufficient magnitude and/or duration to have adversely affected the validity of the study.

B. Protocol

On the day before dosing, the hair of each rabbit was closely shaved to expose the back from the scapular to the lumbar region. The rabbits were then fitted with plastic collars to prevent ingestion of the test substance or disruption of the wrappings.

The test substance was weighed out for each of 2 animals on the day of treatment at a dosage of 5000 mg/kg. The dosages were calculated based on body weights collected prior to treatment. The rabbits' body weights were 2113 or 2187 grams on the day of treatment. The aliquot of the test substance designated for an animal was moistened with approximately 1.0 mL of deionized water to form a thick paste. The paste was spread evenly, directly on the skin, covering an area of approximately 190 square centimeters in size.[1] The test substance was covered with 2-ply gauze. This procedure was repeated for the second rabbit.

After application of the test substance and gauze, the rabbits were then wrapped with successive layers of plastic film, stretch gauze bandage, and elastic adhesive bandage. After wrapping, the rabbits were returned to their cages. Observations for clinical signs of toxicity were conducted approximately 2 hours after dosing. In the absence of visible evidence to the contrary, the test substance was assumed to be stable under the conditions of administration.

Approximately 24 hours after treatment, the rabbits were removed from their cages, and the wrappings were removed. Excess test substance was washed from the animals' backs with warm water, and the skin was dried with a paper towel. Approximately 1 hour after test substance removal, the animals were weighed, observed for clinical signs of toxicity and dermal irritation, and returned to their cages. Observations for dermal irritation were made according to the Draize Scale (Table I). Observations for dermal irritation and clinical signs of toxicity were conducted daily for 14 days after treatment (excluding weekends). Observations for mortalities were made daily throughout the study. The animals were weighed on the day of treatment and on days 1, 7, and 14 following treatment.

C. Records Retention

All raw data and the final report will be stored in the archives of Haskell Laboratory for Toxicology and Industrial Medicine, E. I. du Pont de Nemours and Company, Newark, Delaware or in the DuPont Records Management Center, Wilmington, Delaware.

[1] One-hundred ninety square centimeters is equal to approximately 10 percent of the total body surface of rabbits in the 2-3 kg body weight range.

RESULTS

A. Dosage and Mortality

The dosage regimen and the mortality resulting over the 14-day test period are detailed below. No deaths occurred during the study.

Dosage (mg/kg)	Dose (g)	Initial Body Weight (g)	Mortality
5000	10.94	2187	No
5000	10.56	2113	No

B. Clinical Signs and Dermal Effects

Nonlethal Doses

No clinical signs of toxicity or edema were observed during this study. H-21216 produced moderate erythema in both rabbits by 1 day after application and was still observed 2 and 3 days after application. By 6 days, mild or moderate erythema was observed. Mild erythema persisted from 7 to 10 days. By 13 days after application, the rabbits exhibited slight erythema; all erythema had cleared by study completion. Epidermal scaling and sloughing were observed in both rabbits from 6 to 13 days after application. In addition, one rabbit exhibited a small area of necrosis outside the test site from 2 to 6 days after application; this area of necrosis was attributed to the test substance running outside the test site. By 7 days, the area of necrosis had sloughed and an area of alopecia was then observed until study completion.

Individual body weights are presented in Table II. Individual clinical signs of toxicity are presented in Table III. Individual dermal responses are presented in Table IV.

CONCLUSION

Under the conditions of this study, the ALD for H-21216 was greater than 5000 mg/kg of body weight. This substance is considered to be, at worst, slightly toxic (ALD between 5000 and 10,000 mg/kg) when applied to the shaved, intact skin of male rabbits.

APPROXIMATE LETHAL DOSE (ALD) BY SKIN ABSORPTION OF H-21216

TABLE I

DRAIZE[1] SCALE FOR SCORING SKIN IRRITATION

Evaluation of Skin Reactions	Score
Erythema and eschar formation:	
No erythema	0
Very slight erythema (barely perceptible)	1 (Slight)
Well-defined erythema	2 (Mild)
Moderate to severe erythema	3 (Moderate)
Severe erythema (beet redness) to slight eschar formation (injuries in depth)	4 (Severe)
Edema formation:	
No edema	0
Very slight edema (barely perceptible)	1 (Slight)
Slight edema (edges of area well defined by definite raising)	2 (Mild)
Moderate edema (raised approximately 1.0 mm)	3 (Moderate)
Severe edema (raised more than 1.0 mm extending beyond the area of exposure)	4 (Severe)

Abbreviations of other dermal effects are:

A = Abraded	F = Fissuring	L = Sloughing
I = Intact	N = Necrosis	R = Raw Areas
T = Thickening	G = Fissuring with Bleeding	X = Compound Adhered to Skin
C = Eschar	S = Epidermal Scaling	SN = Superficial Necrosis
- = No Effect		
B = Blanching		

[1] Draize, J. H., "Dermal Toxicity." Appraisal of the Safety of Chemicals in Foods, Drugs and Cosmetics. The Editorial Committee of the Association of Food and Drug Officials of the United States, Austin, Texas, 1959, pp. 46-59.

APPROXIMATE LETHAL DOSE (ALD) BY SKIN ABSORPTION OF H-21216

TABLE II

INDIVIDUAL BODY WEIGHTS (g) OF RABBITS
TREATED WITH H-21216

Rabbit Number	Dosage (mg/kg)	Initial Wt.	Days After Application 1	7	14
30582	5000	2187	2191	2422	2563
30583	5000	2113	2109	2362	2457

APPROXIMATE LETHAL DOSE (ALD) BY SKIN ABSORPTION OF H-21216

TABLE III

INDIVIDUAL CLINICAL SIGNS OF TOXICITY

Clinical Observations

No clinical signs of toxicity were observed in either rabbit during the study.

Individual Skin Responses

ERYTHEMA

Rabbit Number	Days After Application 1	2	3	6	7	8	9	10	13	14
30582	3	3	3	3	2	2	2	2	1	0
30583	3	3	3	2	2	2	2	2	1	0

EDEMA

Rabbit Number	Days After Application 1	2	3	6	7	8	9	10	13	14
30582	0	0	0	0	0	0	0	0	0	0
30583	0	0	0	0	0	0	0	0	0	0

OTHER DERMAL EFFECTS

Rabbit Number	Days After Application 1	2	3	6	7	8	9	10	13	14
30582	-	N	N	N	S,L, A	S,L, A	S,L, A	S,L, A	S,L, A	A
30583	-	-	-	-	S,L	S,L	S,L	S,L	S,L	S,L

N = Area of necrosis (approximately 1 cm x 3 cm)
A = Alopecia observed where necrosis had sloughed.

DUPONT CENTRAL RESEARCH AND DEVELOPMENT
HASKELL LABORATORY FOR TOXICOLOGY
AND INDUSTRIAL MEDICINE

April 1, 1996

TO: MR 10074-001
Approximate Lethal Dose (ALD) by Skin Absorption of H-21216 in Rabbits

FROM: Tracy A. Filliben
Study Director

Notebooks used in this study were:

E-82728
E-82728-FF, pp. 1-11

Distribution of the final report (HLR 839-95) was:

S. R. Laas (1)
P. R. Resnick (1)
F. P. Mulherin (1)
W. J. Brock (1)
L. W. Buxton (1)
J. M. Migliore (1)
W. M. Stewart (1)
R. Valentine (1)
T. A. Filliben (1)

MR Number	10074-001
Haskell Number	21216
Study Code	665
Metrics Report Code	36

Compound Submitter: Paul R. Resnick

==========

**COMPLETE THIS PORTION AFTER REPORT IS SIGNED,
BUT BEFORE REPORT IS SENT TO INFORMATION SERVICES**

Does the summary from this report qualify for the ICARE database? yes (no)

If yes, who made the electronic submittal? _____

If yes, when was the electronic submittal sent? ___/___/___